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EXTRACTION AND ANALYSIS OF SULFUR MUSTARD (HD) FROM VARIOUS FOOD MATRICES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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14. ABSTRACT:
Gas chromatography–mass spectrometry was used to analyze sulfur mustard (HD) in various food matrices. The development of a solid-phase extraction method using a normal-phase silica gel column for the extraction of HD in several food matrices is described. Various concentrations of agent, ranging from 2 to 3 mg, were spiked into food samples. The Agent Chemistry Branch at the U.S. Army Edgewood Chemical Biological Center has developed three extraction methods for use, depending on the matrix. Matrices included orange juice, apple juice, whole milk, 2% milk, Egg Beaters egg whites, tomato sauce, and several meats, including ground beef (80% lean and 20% fat), hot dogs, chicken nuggets, and turkey deli meat (99% fat free). The total percent recoveries (and percent relative standard deviations) for HD in various food samples are reported.

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PREFACE

This work was started in November 2013 and completed in January 2014.

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EXTRACTION AND ANALYSIS OF SULFUR MUSTARD (HD) FROM VARIOUS FOOD MATRICES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

1. INTRODUCTION

Since its introduction on the battlefield in World War I, sulfur mustards, bis(2-chloroethyl) sulfide (HD) and related compounds, have been important chemical warfare agents. In the years since World War I, there have been many suspected and recorded uses of sulfur mustard;[1,2] in the 1980s, it was used during the Iran-Iraq war. The recent utilization of sulfur mustard, combined with its stockpiling by several countries, ease of production, and potential use by terrorists, has resulted in renewed interest and research. A recent search of *Chemical Abstracts*[3] yielded more than 900 references to sulfur mustard in the last five years alone.

The degradation of sulfur mustard in the environment and in storage is complex. The pathways and products of sulfur mustard degradation under a variety of field and laboratory conditions have been extensively described.[4–11] An analysis of sulfur mustard ton containers in the U.S. stockpile showed that in addition to sulfur mustard, byproducts formed during manufacturing, and products also formed from slow degradation reactions within the storage container. Analysis of these degradation products is difficult because of their similarity and the lack of easily distinguishable functional groups.

The existence of these molecules in either the environment or the food supply would indicate a compliance breach, even if the actual chemical warfare agent levels were not high enough to cause personal harm. Although the detection of sulfur mustard adducts or metabolites from environmental or biological samples has been reported,[12–21] literature is limited regarding direct detection of actual mustard gas in food.[22,23]

This document reports results obtained by the Agent Chemistry Branch from the Research and Technology Directorate of the U.S. Army Edgewood Chemical Biological Center (ECBC) in developing new extraction and analytical detection methodologies using gas chromatography–mass spectrometry (GC–MS). The objective of this task was to provide development and laboratory support for the extraction of HD (Figure 1) from various food samples. This included detection and quantitative and qualitative analyses of complex matrices, such as foods with high salt and fat contents. In support of this objective, we examined 10 foods: apple juice, orange juice, whole milk, 2% reduced fat milk, Egg Beaters processed egg whites (ConAgra Foods; Omaha, NE), tomato sauce, precooked turkey deli meat (99% fat free), chicken nuggets, hot dogs, and 80/20 ground beef (80% lean and 20% fat), which represent food types commonly associated with school lunch programs. The food types were chosen based on collaborations and conversations with the U. S. Department of Agriculture, and testing was performed using commercially available columns.

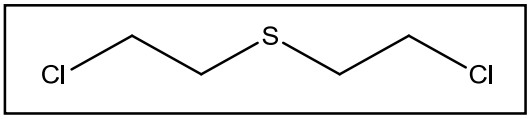

Figure 1. Structure of HD.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

Two structurally identical sulfur mustard blister agents, HD and H, were provided by ECBC. HD is previously distilled mustard; its purity is usually >97%. H is mustard from chemical munitions or ton containers, and it typically contains 20–30% of other sulfur-containing compounds. All reagents and solvents were high-performance liquid chromatography grade. Isopropyl alcohol (IPA) was purchased from Sigma-Aldrich (St. Louis, MO). Apple juice, orange juice, 2% milk, whole milk, Egg Beaters egg whites, tomato sauce, chicken nuggets, 80/20 ground beef , turkey deli meat, and hot dog foodstuffs were purchased from a local grocery store (Food Lion; Edgewood, MD).

2.2 Instrumentation

RediSep Rf normal-phase silica gel columns (5 g) obtained from Teledyne Isco (Lincoln, NE) were used to extract HD from the food samples. GC–MS analysis of HD was performed on an Agilent 5975 mass spectrometer interfaced to a 6890 series gas chromatograph (Agilent Technologies; Santa Clara, CA). The gas chromatograph was equipped with an Agilent J&W Scientific HP-5ms bonded-phase capillary column (30 m × 0.25 mm i.d.) with a film thickness of 0.25 µm. The injection port temperature was 220 °C, the GC–MS interface temperature was 250 °C, and the source temperature was 150 °C. The carrier gas was helium, with a flow rate of 1 mL/min, and the oven temperature was programmed from 60 to 250 °C at 15 °C/min. A split injector was used (split ratio, 75:1), and a 0.2 µL sample volume was placed on the column. The scanned mass range was 50 to 450 Da at 4 scans/s.

2.3 Procedure for HD Extraction from Foodstuffs

Samples of apple or orange juice (2 mL) were placed into glass vials and spiked with 2–3 mg of neat HD. First, the RediSep Rf column (Figure 2) was eluted with 50 mL of 1% diethylmethylamine/2% triethylamine (TEA) in CH_3CN, and in-house air was used to pass the solution through the column. Second, the HD-spiked apple juice was passed through the column, and the sample was collected. Third, 1 mL of 2% TEA in CH_3CN solution was added to the column and pushed slightly into the silica gel, until 1 mL of the solution had just cleared the top of the silica gel. This step was repeated three times. Finally, the remaining 47 mL of 2% TEA in CH_3CN solution was added to the column and passed through the bed. A small aliquot was filtered through a 0.45 µm poly(tetrafluoroethylene) membrane filter and then diluted with IPA (at a 1:10 dilution) for GC–MS analysis.

A similar range of neat HD was spiked into both milk samples. Each milk sample was diluted with 5 mL of CH_3CN. The mixture was centrifuged for 3 min at 10,000 rpm, and the supernatant was decanted. A second 5 mL portion of CH_3CN was added, and the mixture was

vortexed or sonicated for 1 min and again centrifuged for 3 min at 10,000 rpm. The supernatant was removed, and the first and second portions were combined and passed through a RediSep Rf column. The milk sample analysis was performed in an identical manner as described for the juice analysis. The eluents were collected for GC–MS analysis. Samples of approximately 5 g of Egg Beaters egg whites or tomato sauce were spiked with 2–3 mg of neat HD. The sample analyses for the Egg Beaters egg whites and tomato sauce were performed in an identical manner as described for the juice analysis, and the eluents were collected for GC–MS analysis.

A 5 g (±0.1 g) sample of hot dog, turkey deli meat, chicken nuggets, or ground beef was spiked with 2–3 mg of neat HD and diluted with 5 mL of CH_3CN. The entire sample was homogenized using a Polytron homogenizer (Kinematica; Luzern, Switzerland) at 20,000 rpm for 1–2 min. The mixture was then centrifuged for 3 min at 10,000 rpm, and the supernatant was removed. A second 5 mL portion of CH_3CN was added, and the sample was vortexed or sonicated for 1 min and centrifuged for 3 min at 10,000 rpm. The supernatant was removed, and the first and second portions were combined and passed through a RediSep Rf column. The eluents were collected for GC–MS analysis. A total of three food samples were weighed for each matrix, and the percent recoveries for HD with the relative standard deviations (RSDs) were obtained by averaging values from three analysis runs.

Figure 2. A RediSep Rf normal-phase silica gel column.

3. RESULTS AND DISCUSSION

3.1 GC Separation and Analytical Figures of Merit

For GC–MS analysis, the MS system was operated in total ion chromatogram (TIC) mode at mass-to-charge ratio (*m/z)* 50–1200 and single ion monitoring (SIM) mode at *m/z* 159.077. SIM was used to determine the limits of detection and quantitation (LODs and LOQs, respectively) and the linear dynamic ranges (LDRs) for HD. The calibration curve for HD was plotted over a concentration range of 1.0 ng/mL to 8.0 µg/mL, with 1 µL injections at each concentration level. To calculate the LODs for the nerve agents, 1 µL injections were used at HD

concentrations as low as 1 ng/mL, with a signal-to-noise ratio of 3:1. The LOQs for the analyte were also calculated, with a signal-to-noise ratio of 10:1. The linear regression equations were calculated by a least-squares analysis of the LDRs, LODs, and LOQs. The linear regression equations and the correlation coefficients are tabulated in Table 1.

Table 1. Analytical Figures of Merit for HD

Agent	LDR (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Correlation Coefficient[a]
HD in IPA	1.1–7340	1.15	4.59	0.9980

[a]Calculated over the calibration range 0.0011–7.3 µg/mL for HD.

3.2 Extraction of HD from Foodstuffs

An electron impact (EI) mass spectrum usually contains the molecular ion, $M^{+\cdot}$, and many fragment ions, which make EI useful for structural characterization. In this study, we examined the extraction efficiency of HD from 10 different matrices. Samples of apple and orange juices, 2% and whole milk, Egg Beaters egg whites, tomato sauce, chicken nuggets, 80/20 ground beef, turkey deli meat, and hot dogs were tested. To optimize the extraction efficiency of HD, several extraction solvents were examined. The best performance was achieved using a 2% TEA/acetonitrile solution. The extracted samples were then diluted 1:10 with IPA for GC–MS analysis.

Representative GC chromatograms for extracted HD samples that were obtained using the normal-phase silica gel column method are shown in Figures 3–13. For HD in IPA, the HD peak (Figure 3a) eluted at 5.2 min and exhibited $[M^{+\cdot}]$ at *m/z* 158 and loss of Cl^- at *m/z* 123 (Figure 3b). Figures 4–13 show representative gas chromatograms for HD extracted from various food samples, and Figure 8a shows the corresponding mass spectra. HD extracted from the hot dog sample showed two peaks, at retention times R_t1 = 5.07 min and R_t2 = 5.2 min. The peak at R_t1 = 5.07 min was identified as benzoic acid, which was eventually traced back to the hot dog casing. Figure 8c shows the mass spectrum for benzoic acid. Percent recoveries were calculated based on an external calibration curve for HD (Figure 14). The recoveries from the various food samples were consistently greater than 80% (Table 2).

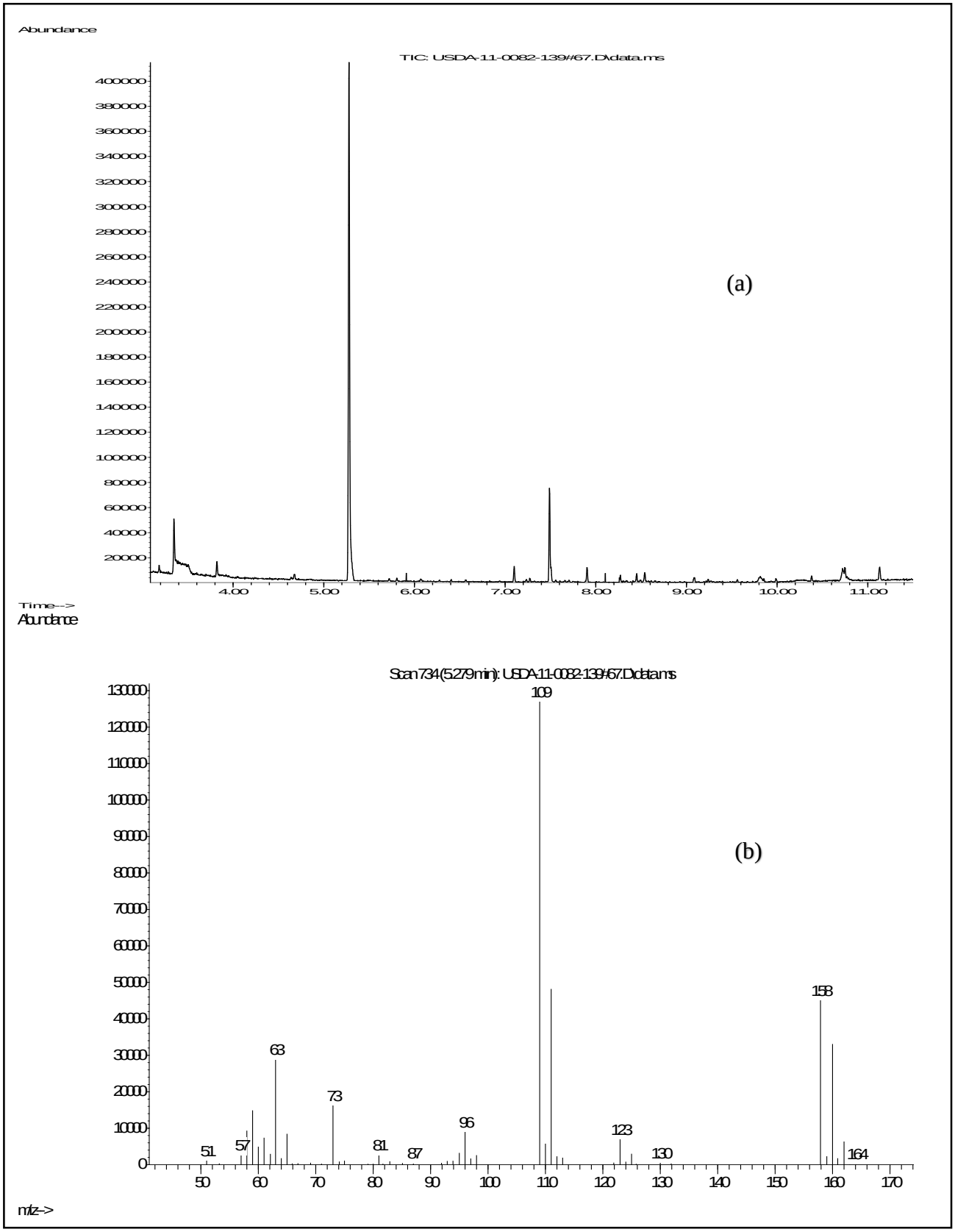

Figure 3. (a) GC chromatogram and (b) mass spectrum for HD standard in IPA.

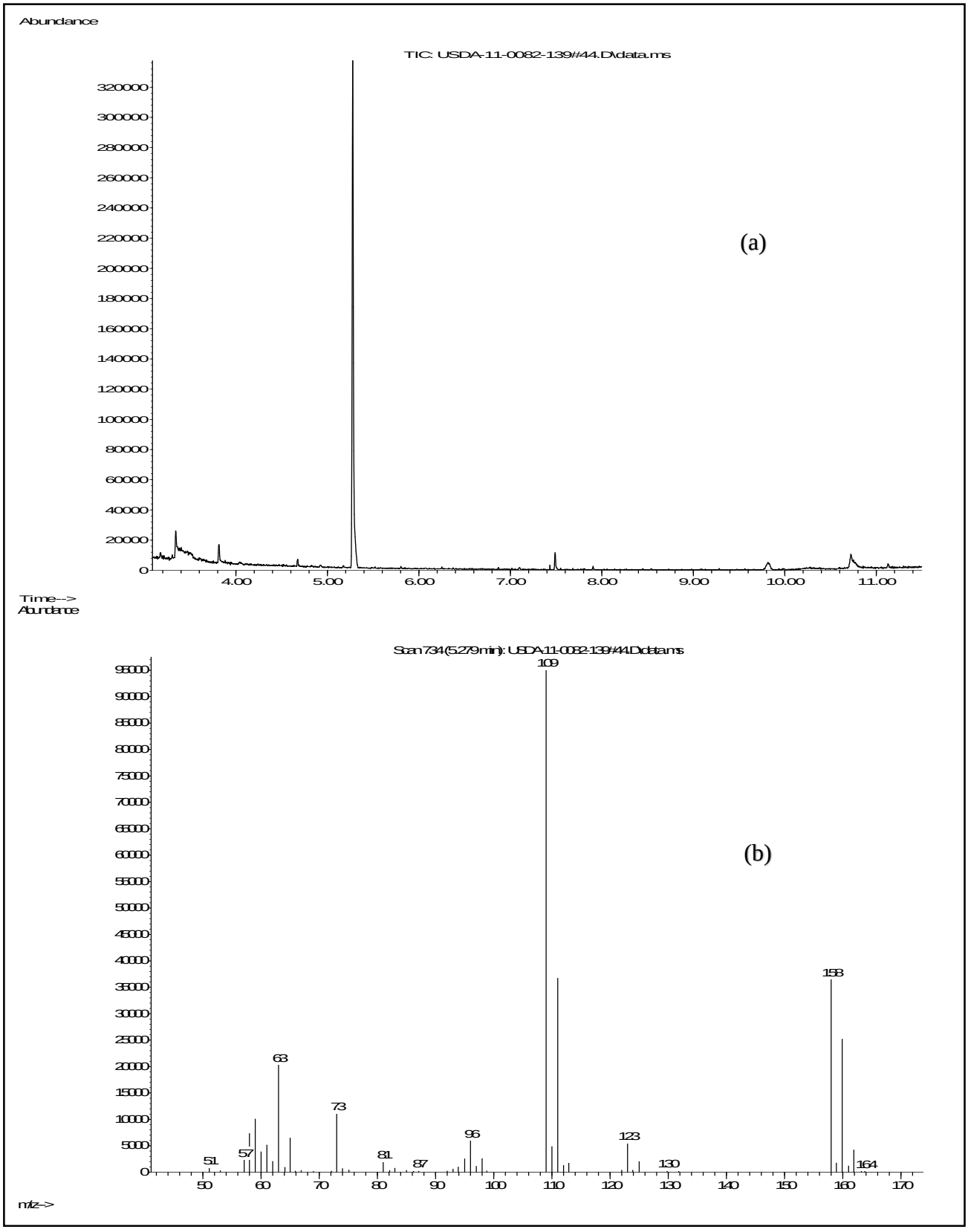

Figure 4. (a) GC chromatogram and (b) mass spectrum for HD extracted from apple juice.

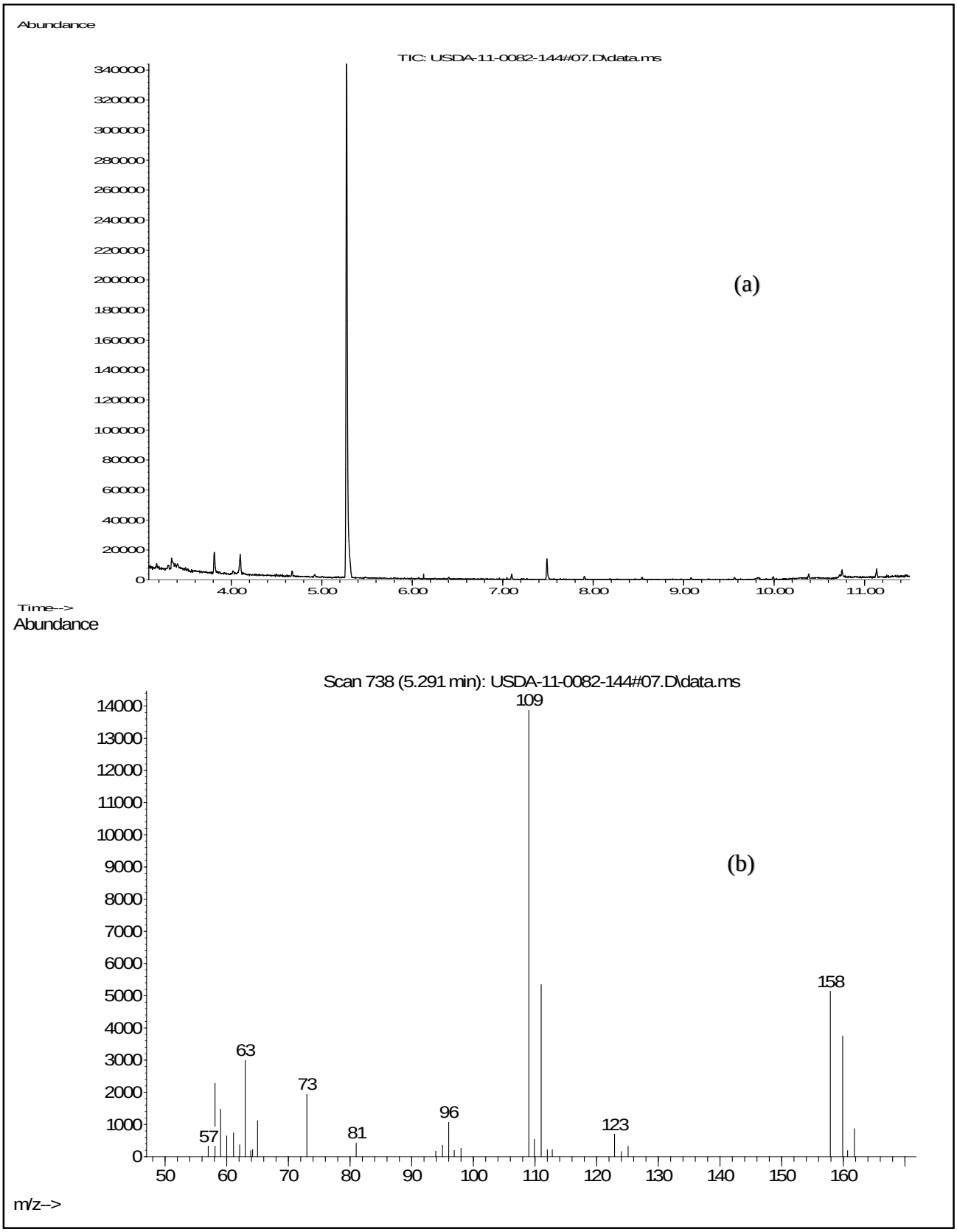

Figure 5. (a) GC chromatogram and (b) mass spectrum for HD extracted from orange juice.

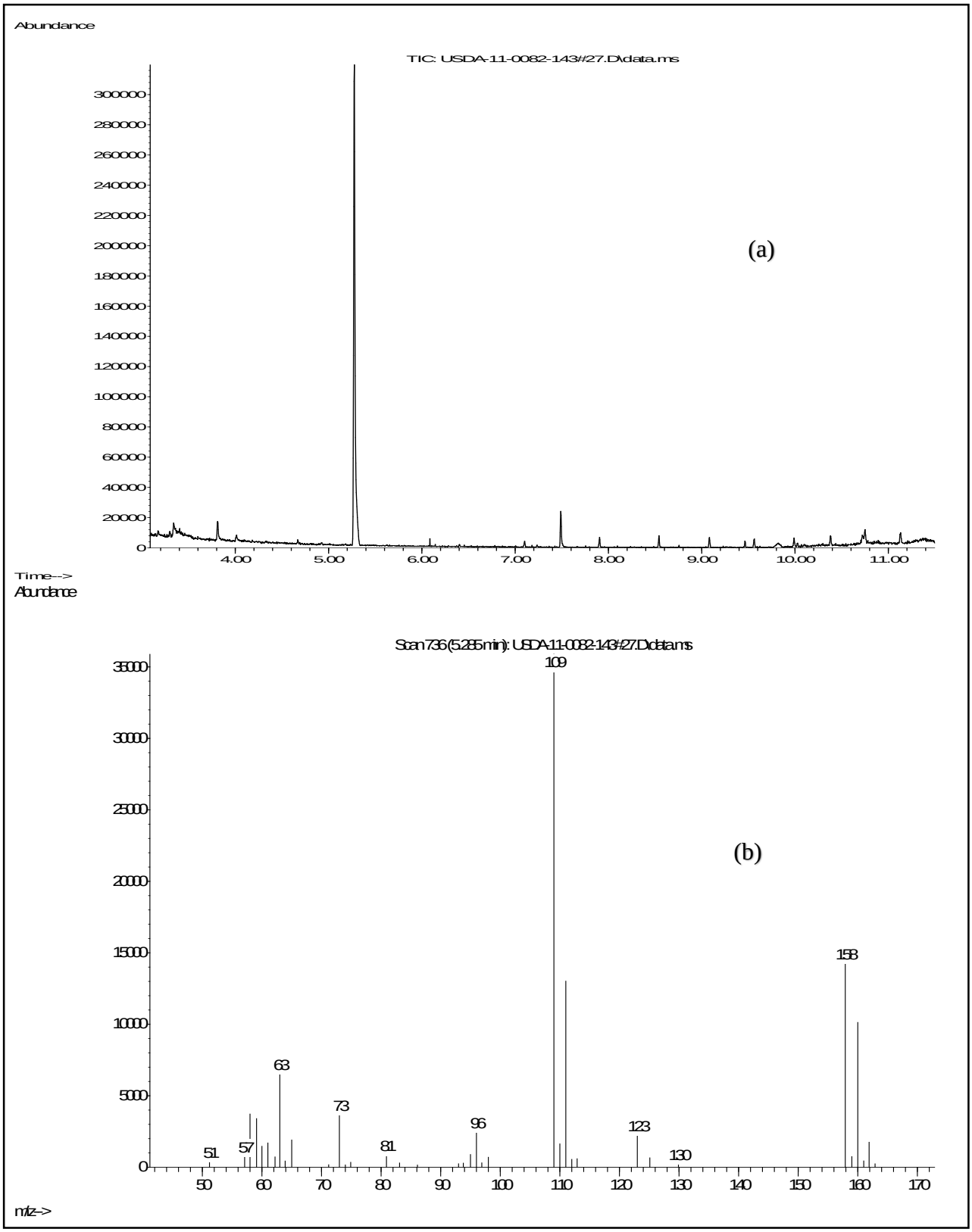

Figure 6. (a) GC chromatogram and (b) mass spectrum for HD extracted from 2% milk.

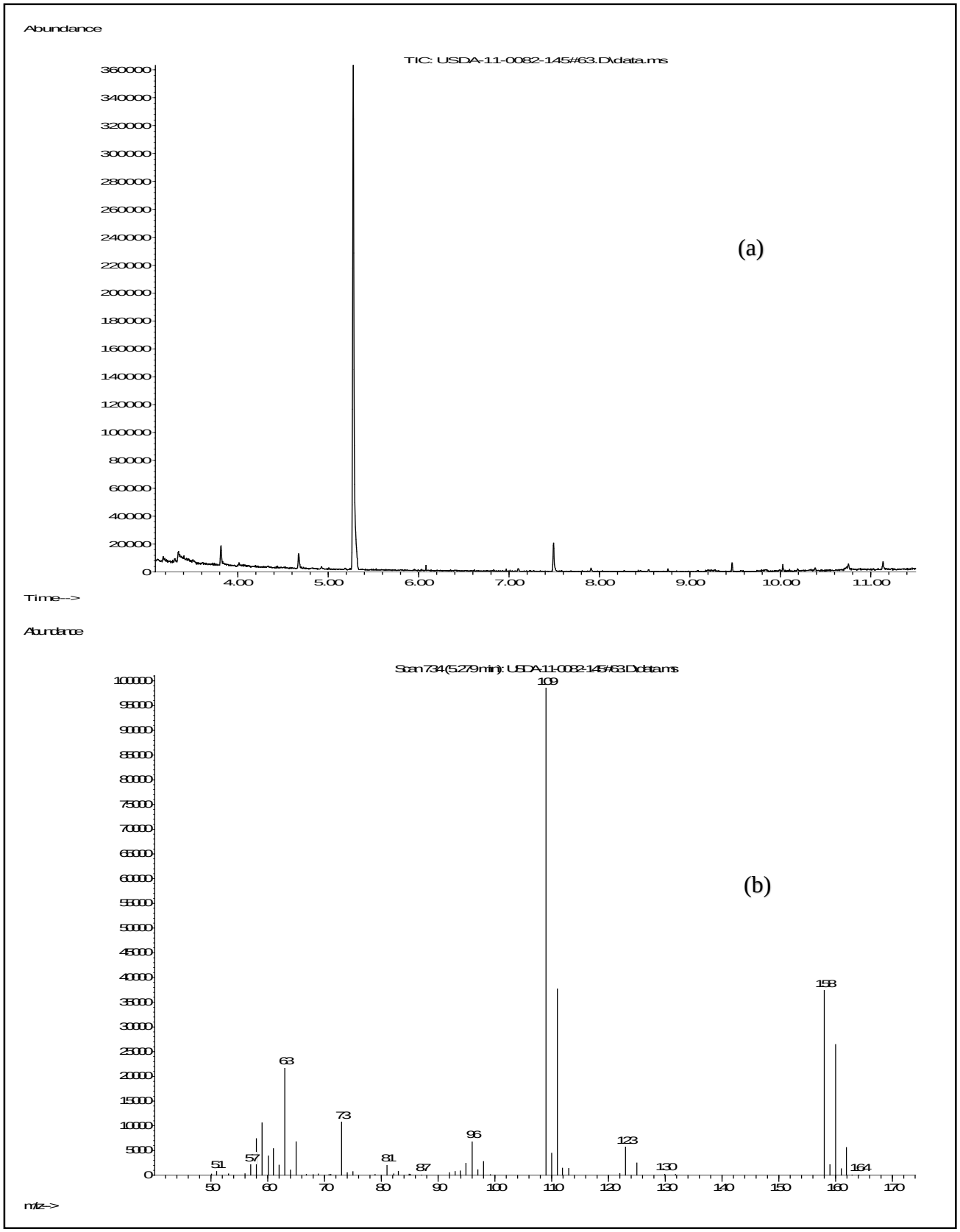

Figure 7. (a) GC chromatogram and (b) mass spectrum for HD extracted from whole milk.

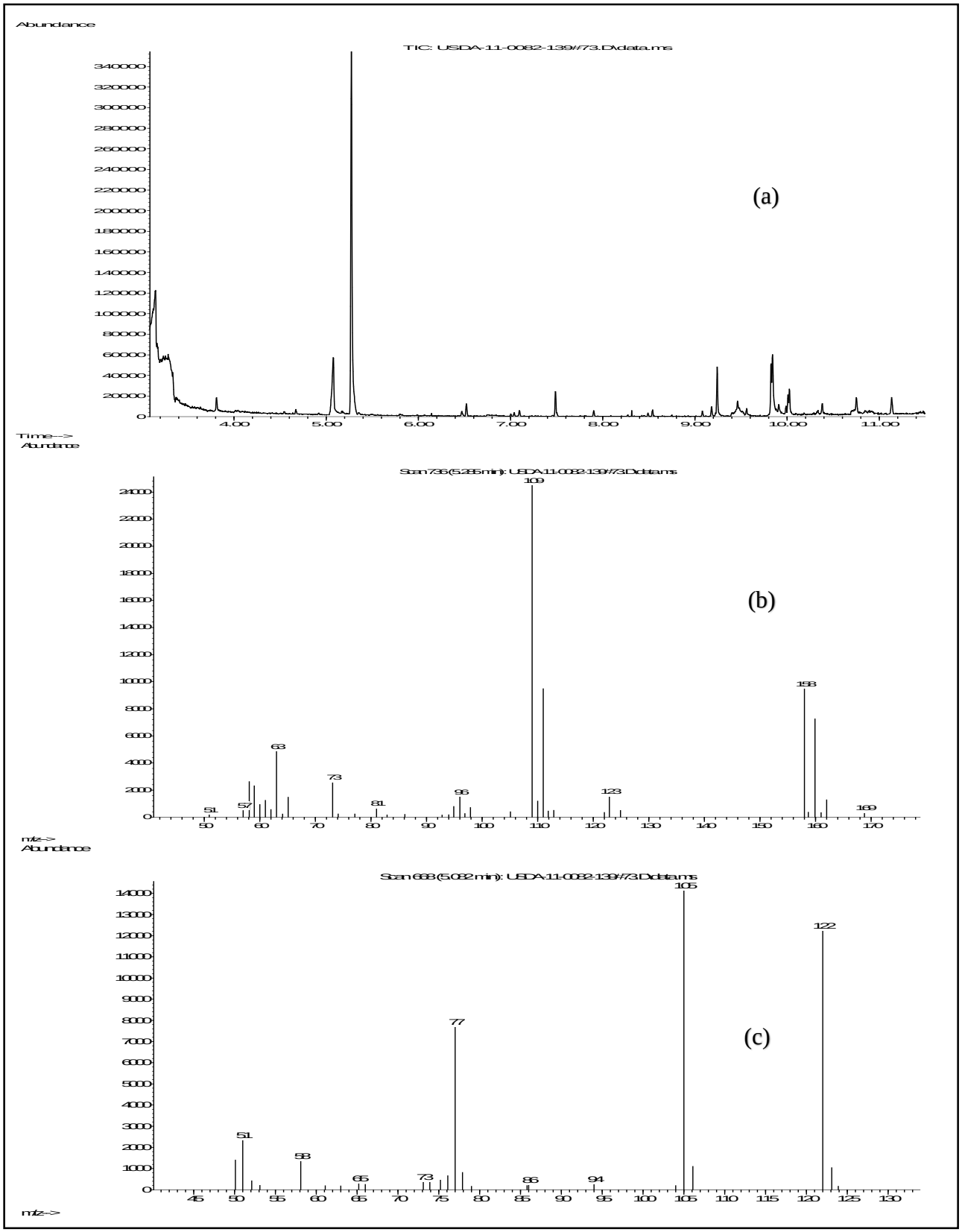

Figure 8. (a) GC chromatogram and (b) mass spectrum for HD extracted from hot dog; (c) mass spectrum at R_t = 5.08 min (benzoic acid).

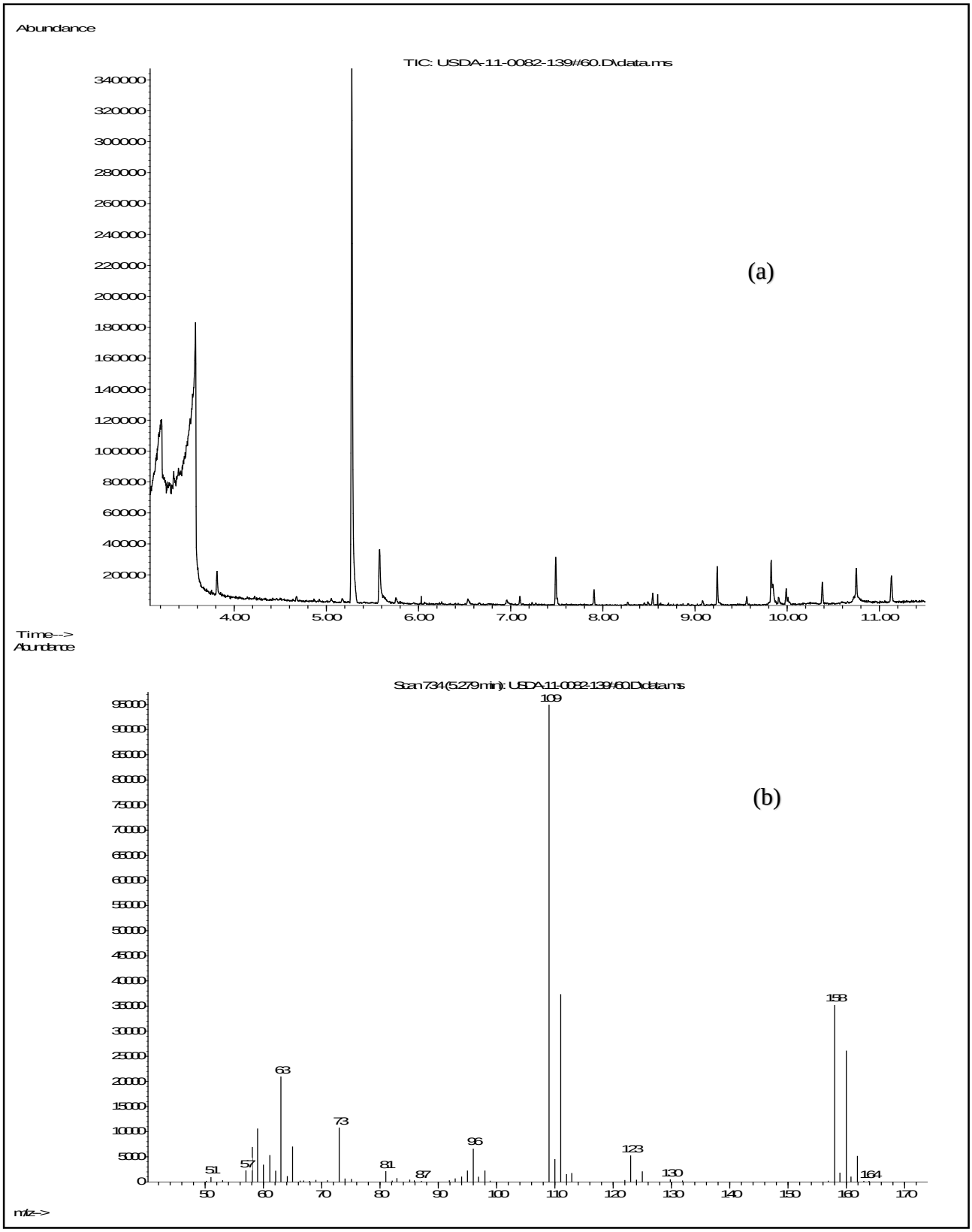

Figure 9. (a) GC chromatogram and (b) mass spectrum for HD extracted from tomato sauce.

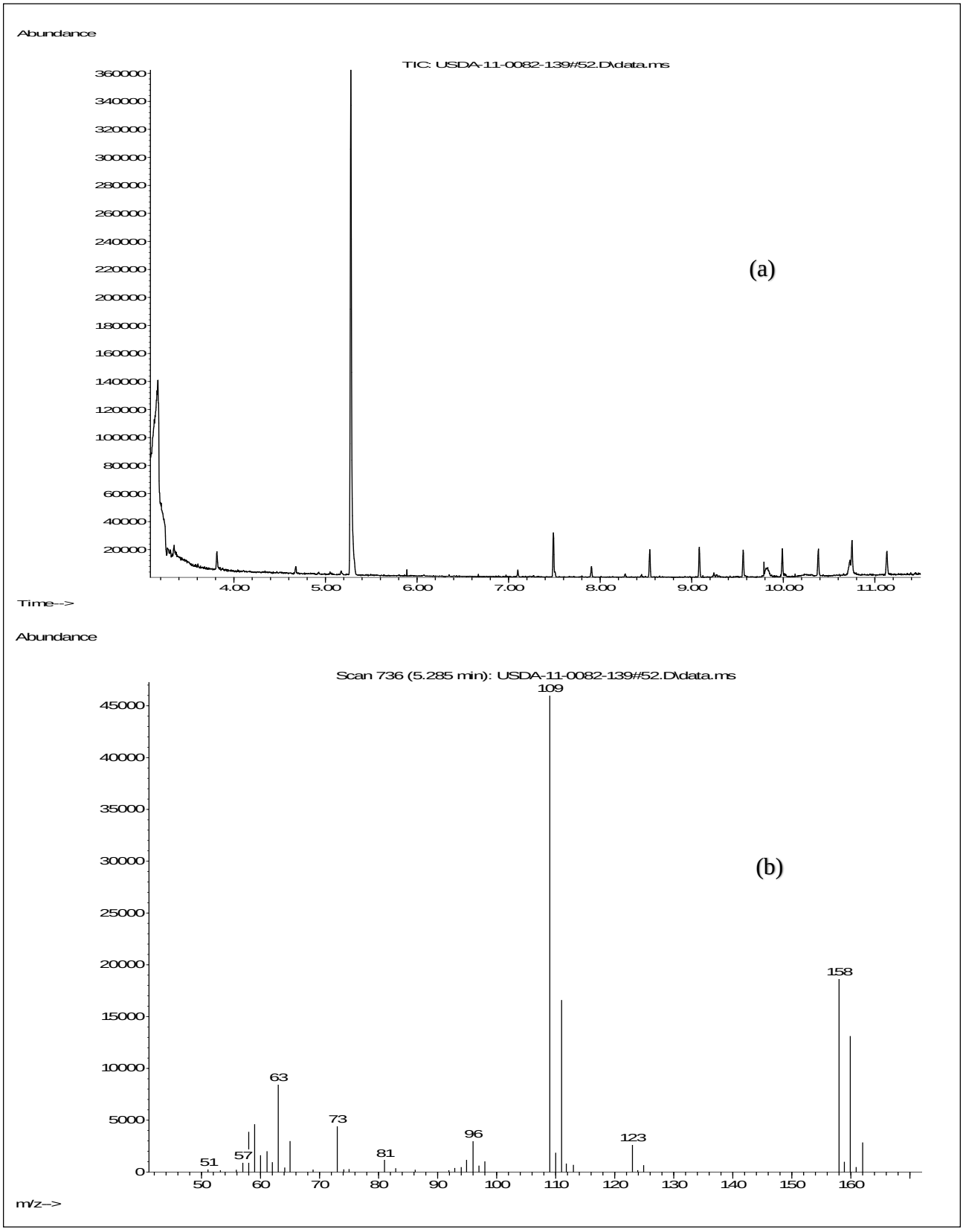

Figure 10. (a) GC chromatogram and (b) mass spectrum for HD extracted from Egg Beaters egg whites.

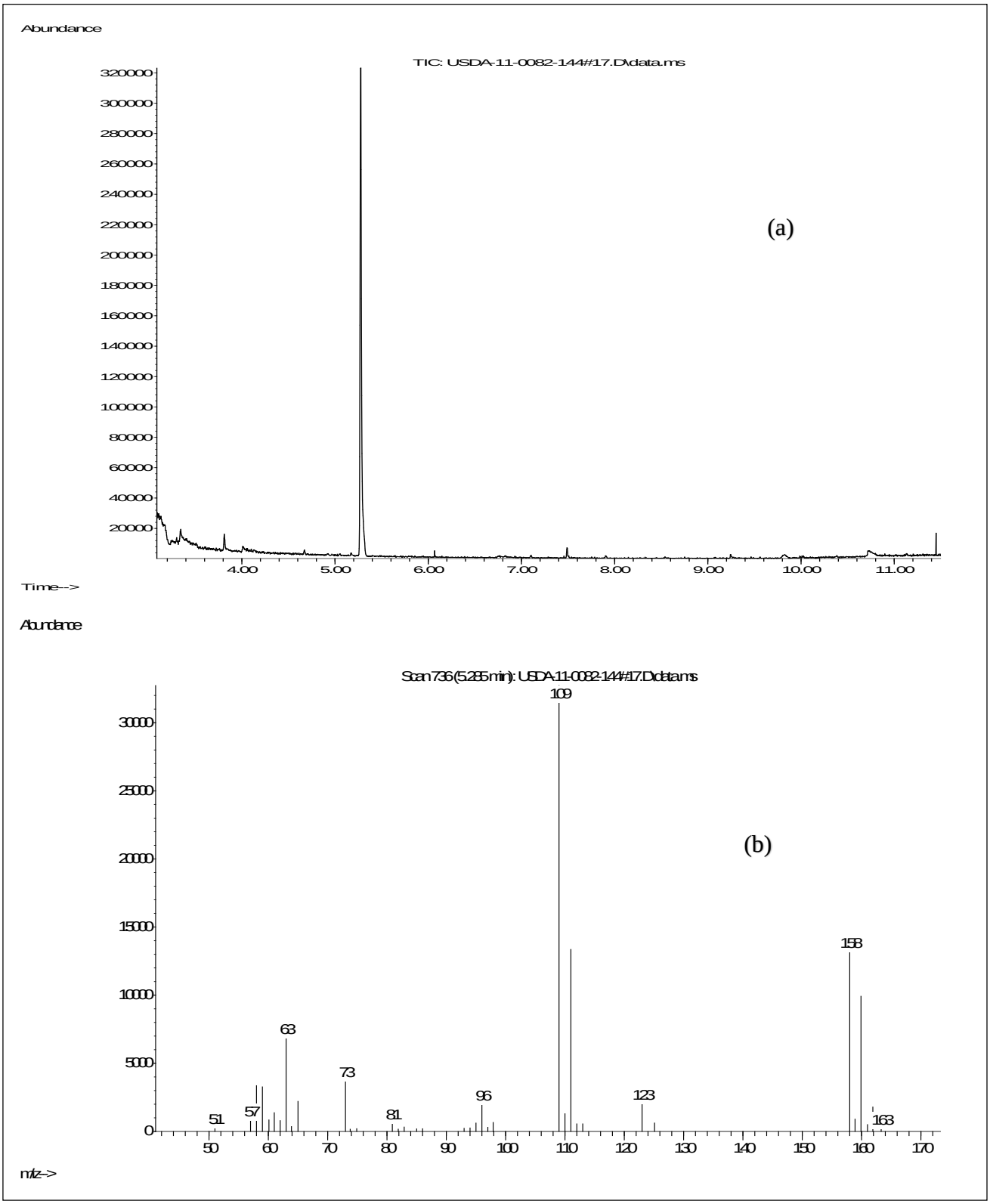

Figure 11. (a) GC chromatogram and (b) mass spectrum for HD extracted from turkey deli meat.

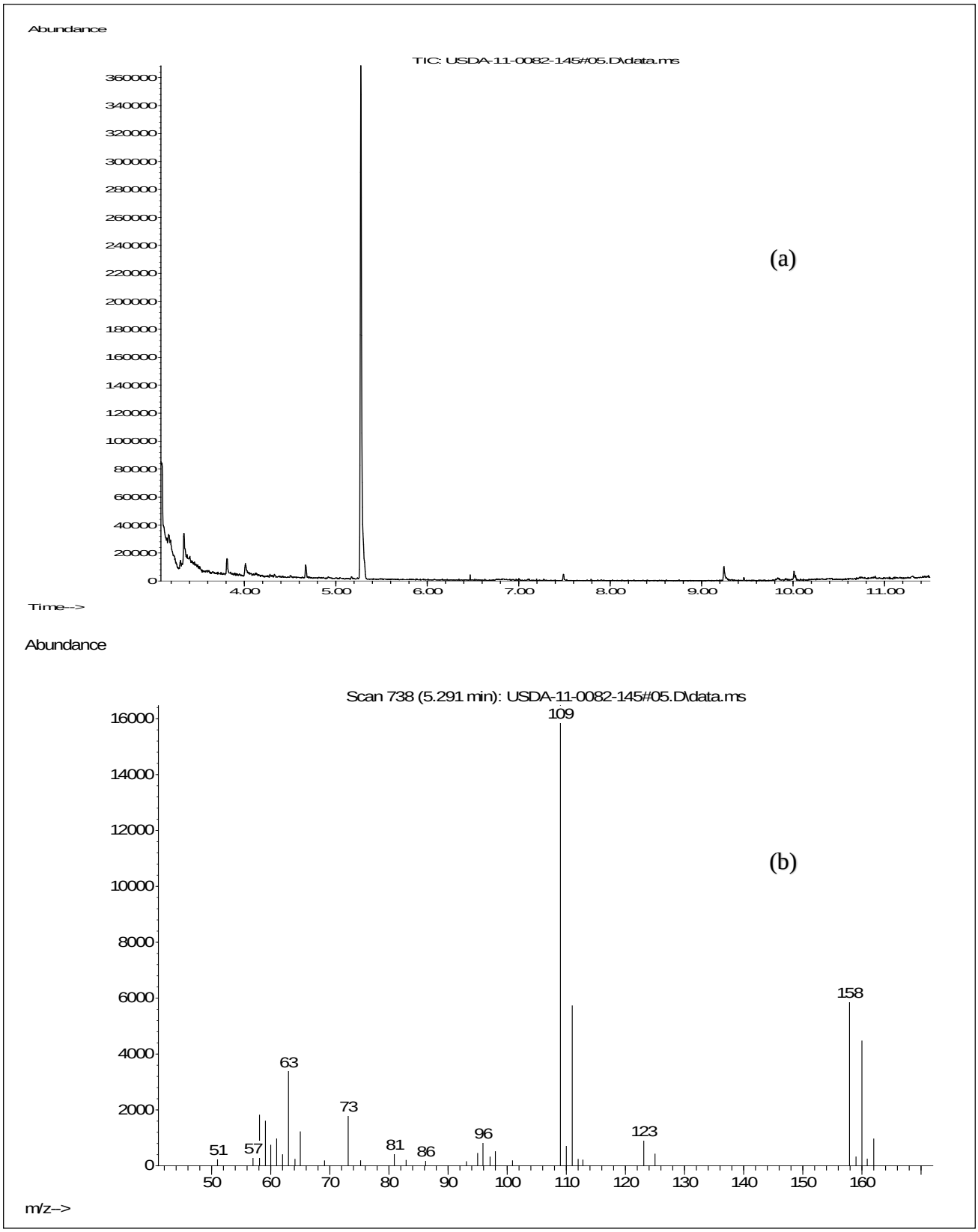

Figure 12. (a) GC chromatogram and (b) mass spectrum for HD extracted from chicken nuggets.

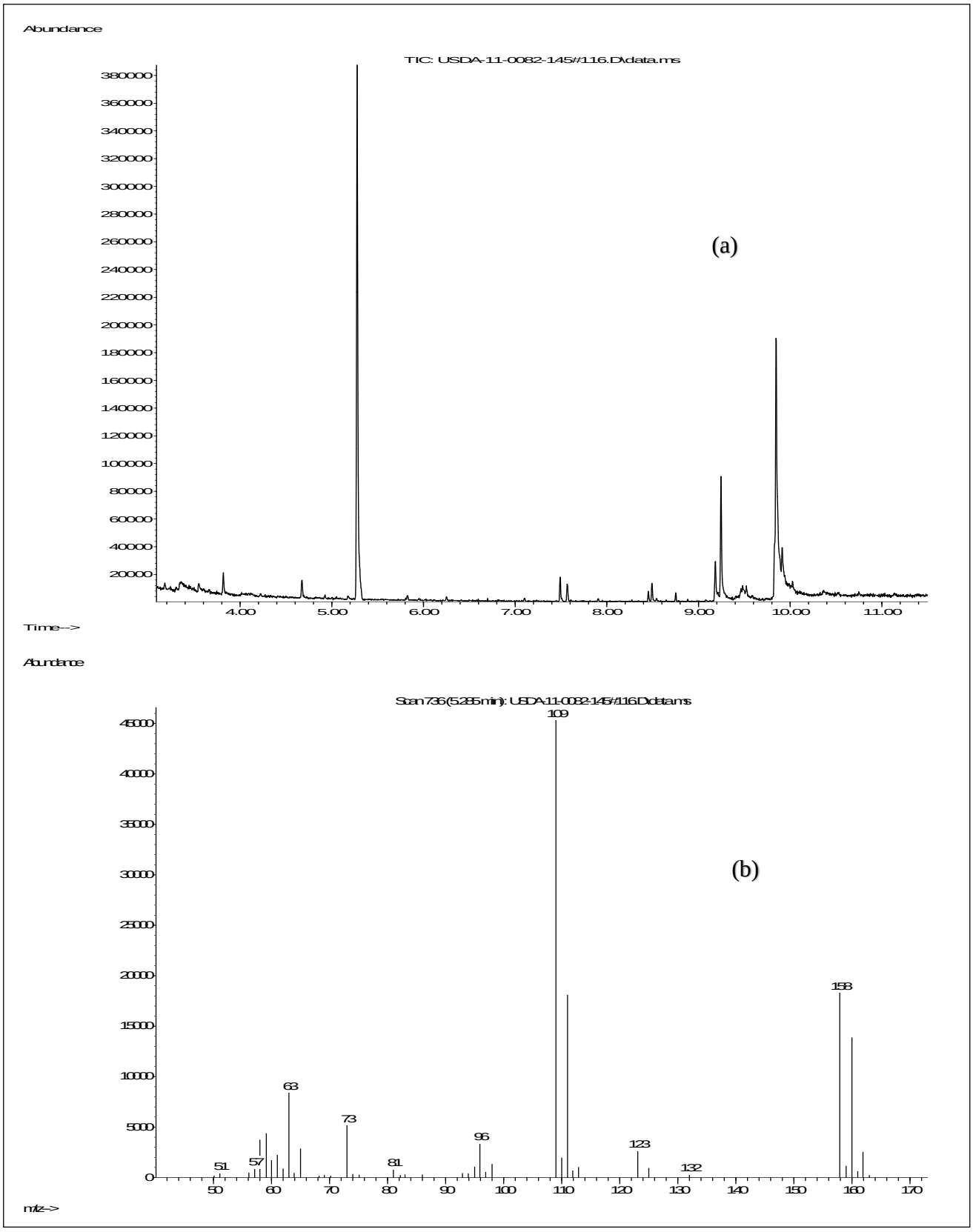

Figure 13. (a) GC chromatogram and (b) mass spectrum for HD extracted from 80/20 ground beef.

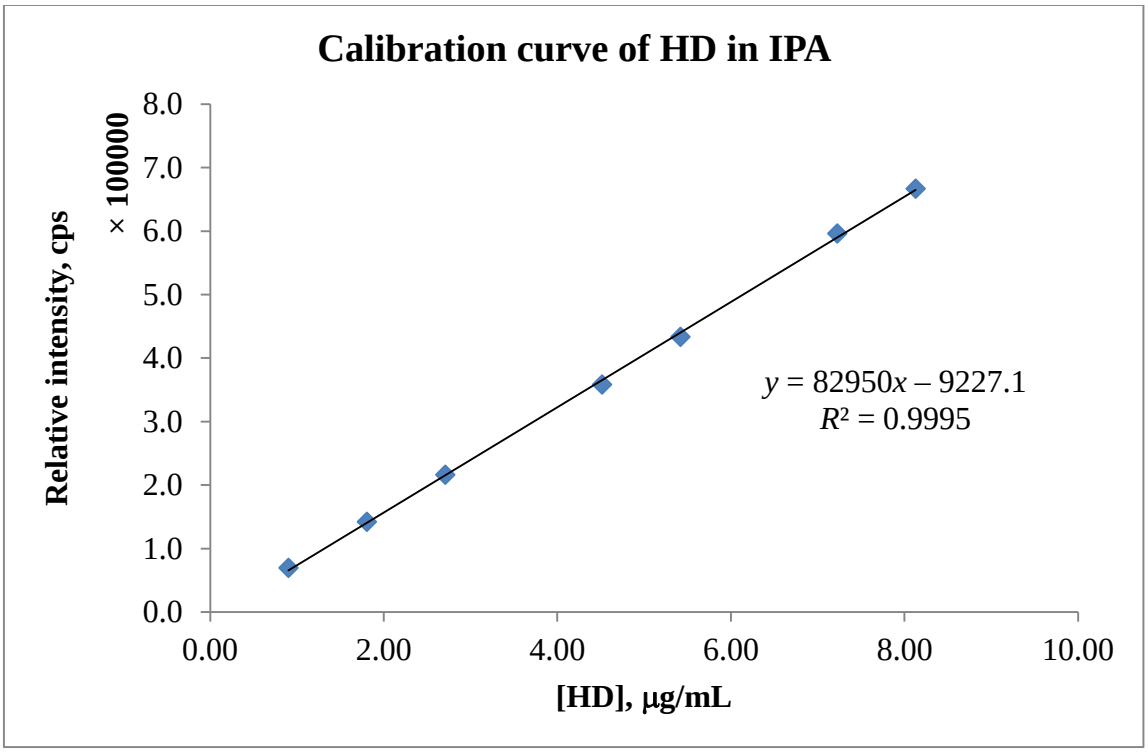

Figure 14. External calibration curve for HD in IPA.

Table 2. HD Extraction Results for Various Food Matrices

Foodstuff	Recovery (%)	RSD (%)
Apple juice	82.5	1.01
Orange juice	82.6	2.27
Whole milk	86.6	2.00
2% milk	91.4	2.23
Egg beaters egg whites	98.0	2.18
Tomato sauce	94.5	3.39
Chicken nuggets	95.4	0.47
80/20 ground beef	89.8	3.12
Turkey deli meat	97.2	0.61
Hot dog	99.3	0.86

Note: Values are averages from three analyses.

3.3 Extraction of H from Munitions-Grade Mustard

Four samples (apple juice, whole milk, tomato sauce, and hot dog) were selected to see how much H could be extracted from food spiked with munitions-grade mustard (MGM). The percentage of H in MGM usually ranges from 80 to 85%, with 1,4-dithiane and sesquimustard being the two other major components present. A control study was performed by dissolving MGM in IPA and then applying the previously described extraction procedure (Section 2.3). The H recovery from this sample was 85%. Approximately 6–7 mg of MGM was spiked into the individual food samples. After workup, the samples were analyzed using GC–MS. Sample quantities included 2 mL of apple juice, 2 mL of whole milk, 5 g of tomato sauce, and 5 g of hot dog. Percent recoveries were calculated based on an external calibration curve for HD. For all of food samples tested, H recovery was greater than 80% (Table 3).

Figures 15 and 16 show representative GC chromatograms for H extracted from MGM-spiked food matrices using the normal-phase silica gel column method. The H peak appeared at 6.74 min and exhibited [$M^{+\cdot}$] at *m/z* 158 and loss of Cl^- at *m/z* 123 (Figure 15). As shown in Figure 16, the first peak at R_t = 5.86 min was identified as 1,4-dithiane, and the third peak at R_t = 10.2 min was identified as sesquimustard.

Table 3. Percent Recoveries and RSDs for H from MGM

Sample	Recovery of H from MGM (%)	RSD (%)
Control: MGM in IPA	85	N/A
Apple juice	80	1.3
Whole milk	86	2.0
Tomato sauce	95	4.6
Hot dog	98	0.8

Note: Values are averages from three analyses.
N/A, not applicable.

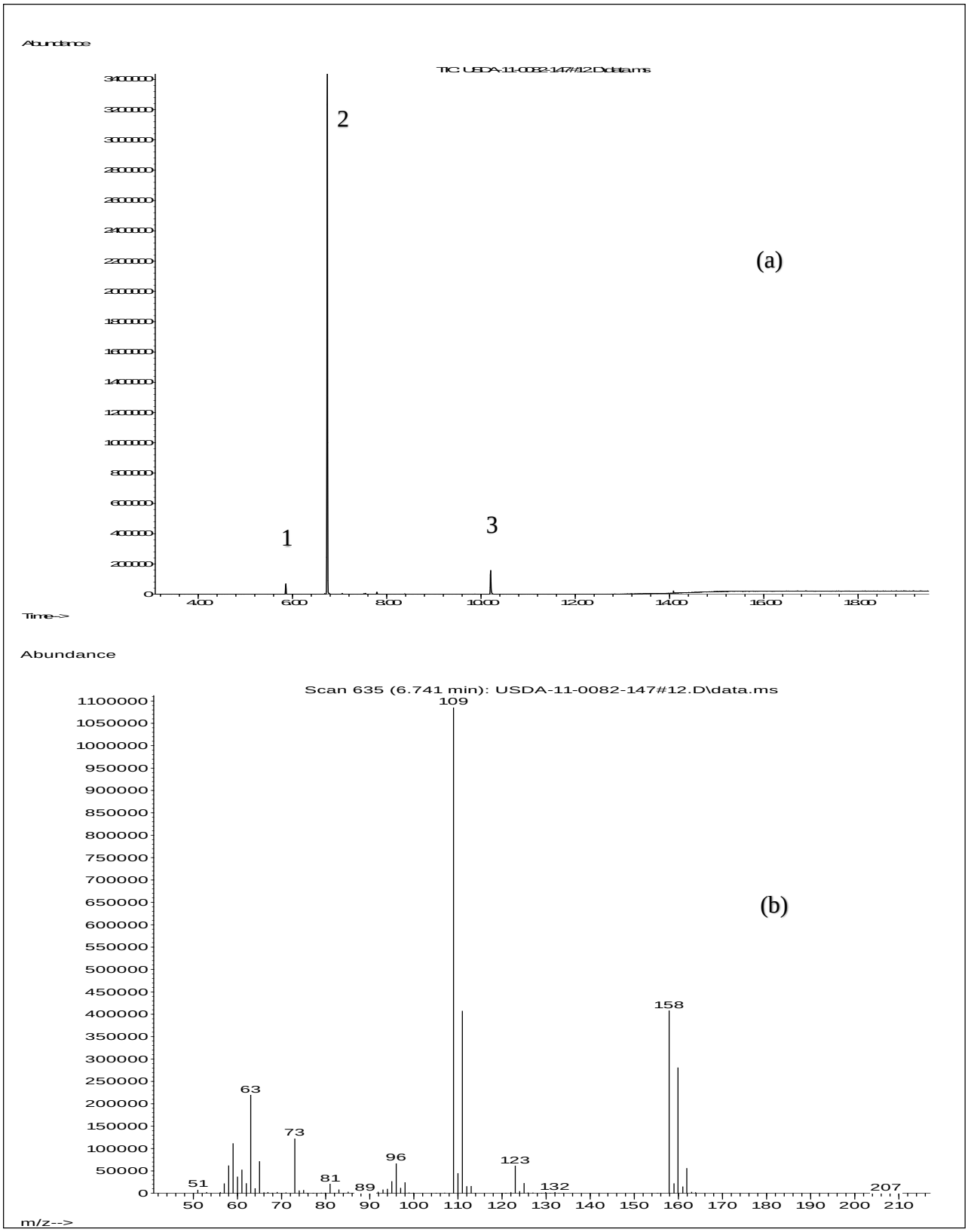

Figure 15 (a) GC chromatogram and (b) mass spectrum for H extracted from MGM (peak no. 2).

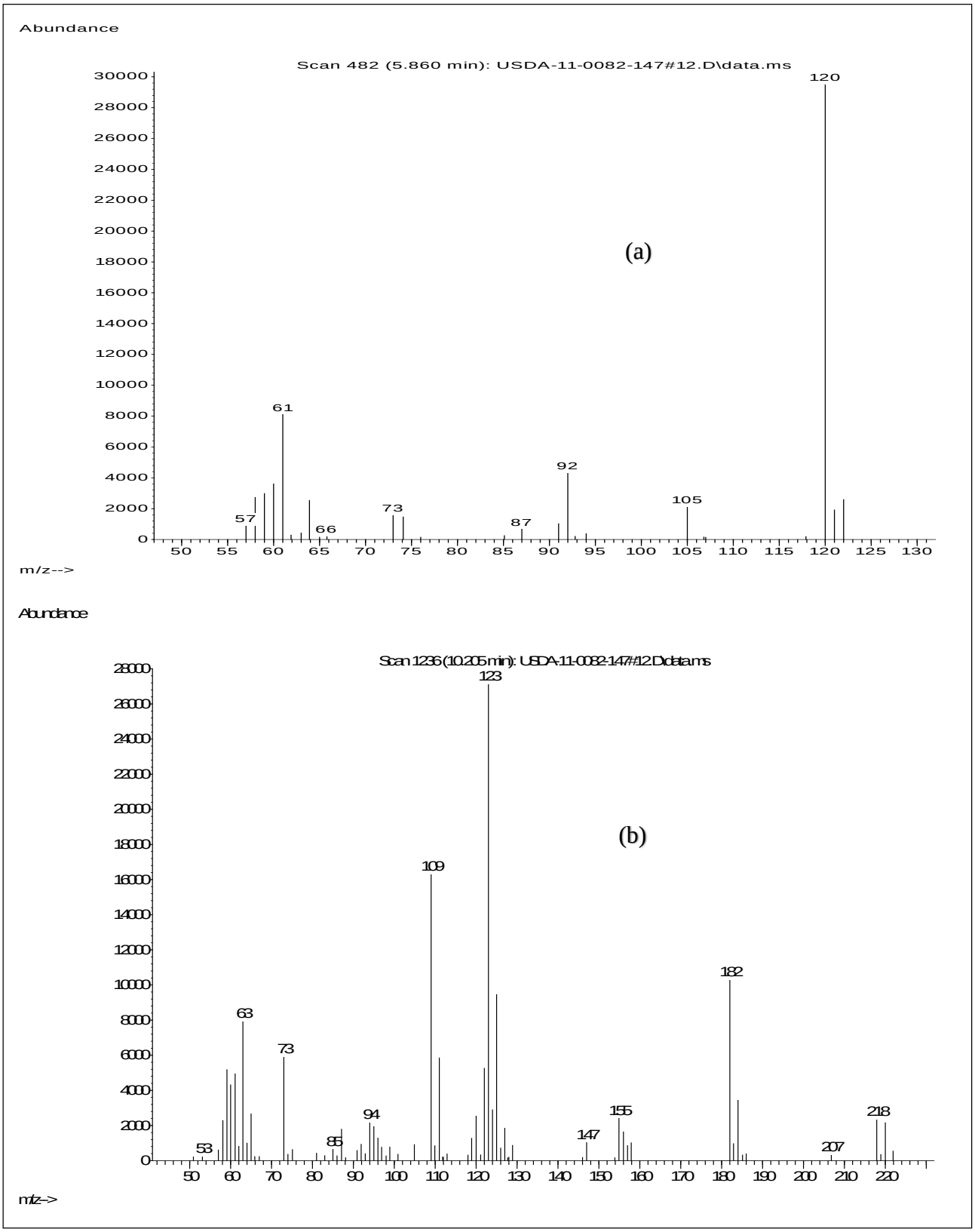

Figure 16. Mass spectra for H extracted from MGM: (a) peak no. 1, for 1,4-dithiane; and (b) peak no. 3, for sesquimustard.

4. CONCLUSION

An extraction technique for HD was successfully developed, and recoveries were greater than 80% for all food matrices. This report details the extraction procedure and the analysis to demonstrate how these results were achieved. This easy-to-use extraction method can be used to determine HD amounts in complex food matrices, including foods in high-salt and high-fat categories. Future work will focus on applying smaller HD spikes to foodstuffs in an effort to use other commercially available columns.

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ACRONYMS AND ABBREVIATIONS

80/20	80% lean and 20% fat
ECBC	U.S. Army Edgewood Chemical Biological Center
EI	electron impact
GC	gas chromatography
HD	sulfur mustard; bis(2-chloroethyl) sulfide
IPA	isopropyl alcohol
LDR	linear dynamic range
LOD	limit of detection
LOQ	limit of quantitation
$M^{+\cdot}$	molecular ion
MGM	munitions-grade mustard
MS	mass spectrometry
m/z	mass-to-charge ratio
RSD	relative standard deviation
SIM	single ion monitoring
TEA	triethylamine
TIC	total ion chromatogram

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